RESEARCH ARTICLE

# Effect of Cinnamon (*Cinnamomum zeylanicum*) Essential Oil as an Alternative to Antibiotic Growth Promoter in Broilers

Devasi M. Solanki[1]*, Chandrakant J. Dave[1], Bharat B. Bhanderi[2], Aaftabhussain L. Sheth[1], Dinesh J. Ghodasara[1]

## Abstract

The present investigation was planned to evaluate the effects of cinnamon oil as an alternative to antibiotic growth promoter in broilers. A total of 144 broiler chicks were randomly allocated to six experimental groups, each consisted of four replicates and each replicate consisted of six chicks. The experimental groups were (I) basal diet; (II) basal diet + chlortetracycline @ 1 g/kg feed; (III) basal diet + cinnamon oil @ 400 mg/kg feed; (IV) basal diet + *E. coli* @ 1.0 mL ($1.5\times10^8$ CFU/mL) orally on 14$^{th}$ day; (V) basal diet + chlortetracycline + *E. coli*; and (VI) basal diet + cinnamon oil + *E. coli*. The study was conducted for 28 days. There was significant decrease in *E. coli* counts ($Log_{10}$ CFU/g) in precaecal-caecal digesta of birds supplemented with cinnamon oil (group III) and chlortetracycline (group II) as compared to other groups on 21$^{st}$ and 28$^{th}$ day of experiment. The caecal average bacterial count of *E. coli* in birds of group-V and group-VI on 7$^{th}$ day post-infection was significantly higher than group-I, while significantly lower than group-IV. However, on 14$^{th}$ day post-infection the caecal average bacterial count of *E. coli* in birds of group-V and group-VI was significantly lower than group-I. It was noticed that supplementation of chlortetracycline was more effective than cinnamon oil as an antibacterial agent. There was significant increase in one or all measurements, such as villi height, villi width or villi height to crypt depth ratio in the duodenum, jejunum and ileum of birds supplemented with cinnamon oil (group III) as compared to the birds of other groups on 21$^{st}$ and 28$^{th}$ day of experiment. The overall gross and histopathological lesions in birds of *E. coli* infected groups were mild degenerative changes in small intestine on 21$^{st}$ day of sacrifice. Based on foregoing observations, it can be summarized that the cinnamon oil can be used by the poultry farmers as an alternative to antibiotic growth promoter.

**Keywords:** Antibiotic growth promoters, Broiler chicken, Cinnamon oil, Colony forming unit, *Escherichia coli,* Gross and histopathology, Gut morphometry.

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## Introduction

The ban on antibiotic growth promoters (AGPs) as poultry feed additives in many countries including India has compelled researchers to find non-therapeutic and safe alternatives to AGPs. To be an ideal antibiotic alternative, the new feed additives must have the same benefits as AGPs in terms of increasing digestibility, ensuring proper growth performance, and improving immunity. Many reports suggest that potential feed additives, such as probiotics, phytogenics, enzymes, direct-fed microbials (DFM), bacteriocins, antimicrobial peptides, yeast-derived components *etc.* could be used as an alternative to AGPs (Manafi *et al.,* 2016). Phytogenics, especially essential oils (EOs) are a group of natural growth promoters that have gained more attention in recent years as an alternative to AGPs. Various plant extracts are used as feed additives in the poultry industry among which cinnamon essential oils (CEOs) have become more interesting due to their potential antibacterial effects as compared to other phenolic oils such as carvacrol and thymol (Di Pasqua *et al.*, 2006; Zhou *et al.*, 2007).

Cinnamon (*Cinnamomum zeylanicum*), one of the oldest medicinal plants and widely used around the world, can be used in poultry rations in the form of powder or essential oil. *Cinnamomum zeylanicum* (bark and leaf oil) and *Cinnamomum cassia* (cassia oil) are the most important volatile cinnamon oils. These oils and their components (cinnamaldehyde and eugenol) possess antibacterial action against *Pseudomonas aeruginosa*, *Salmonella* spp., *Enterococcus faecalis*, *Staphylococcus* spp., *Parahaemolyticus* (Chang *et al.,* 2001), *Escherichia coli* and *Clostridium* spp. (Chowdhury *et al.*, 2018). Moreover, it has a high coliform inhibitory ability in gastrointestinal tract and

[1]Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand-388001, Gujarat, India.

[2]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand-388001, Gujarat, India.

**Corresponding Author**: Devasi M. Solanki, Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Anand-388001, Gujarat, India., e-mail: devasisolanki9@gmail.com

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this antimicrobial ability selectively increases the growth of beneficial bacterial species, such as lactobacilli (Chowdhury *et al.*, 2018). Therefore, addition of EOs in poultry ration could be a potential alternative to AGPs and also an environmentally safe solution. Various researchers around the world are trying to characterize a range of biological properties of essential oils which include antimicrobial, antioxidant, antiviral, immunomodulatory and anti-inflammatory (Bakkali *et al.*, 2008). Therefore, the aim of the present study was to examine the effect of cinnamon oil as an alternative to AGPs in broiler chicken with or without a challenge of *Escherichia coli*.

## Materials and Methods

### Experimental Birds, Feeding, Housing and Management

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee of the College, AAU, Anand (India), and was undertaken as per the guidelines of the CPCSEA, Government of India, New Delhi. The experiment was conducted on day-old Vencobb broiler chicks from a single hatch. The birds were kept on deep litter system and were reared in standard management practices and monitored daily for any abnormal clinical signs, physical or behavioural changes and mortality if any.

According to the age of the birds, all the birds were fed standard broiler feed, *i.e.,* 0-10 days (pre-starter), 11-20 day (starter) and 21-28 day (finisher) in mash form. The cinnamon oil and chlortetracycline powder were mixed daily at the time of feed offered to make different treatment diets. Throughout the experiment, all birds had *ad libitum* access to clean, fresh, cool drinking water. The birds were protected against various diseases by vaccination and strict biosecurity measures.

### Experimental Design

The experiment was conducted on 140, day-old Cobb-400 broiler chicks. The chicks were randomly allocated to six experimental groups, each consisting of four replicates and each replicate consisting of six chicks. The group-I chicks served as a control and were given basal diet without administration of any treatments. Chicks of group-II were given chlortetracycline (CT Star) as an antibiotic growth promoter @ 1 g/kg feed as a standard control. Chicks of group-III were given cinnamon oil @ 400 mg/kg feed as a treatment control. Chicks of group-IV served as a negative control and given basal diet and challenged with *E. coli* @ 1.0 ml ($1.5\times10^8$ CFU/mL) orally on $14^{th}$ day. Chicks of group-V and VI were given chlortetracycline (1 g/kg feed) and cinnamon oil (400 mg/kg feed), respectively, with *E. coli* ($1.5\times10^8$ CFU/mL) orally on $14^{th}$ day of experiment.

### Assessment of Antibacterial Effect

For this study, *E. coli* isolates from localized and systemic diseases of birds were isolated at the Department of Veterinary Pathology, Veterinary College, Anand.

### Oral Challenge with *E. coli*

*E. coli* isolates were inoculated on EMB (Eosin Methylene Blue) agar and incubated at 37°C for 18 h. A loopful of colonies from this plate was inoculated into BHI (Brain Heart Infusion) broth and was incubated for 12 h at 37°C. The concentration of bacterial suspension was adjusted to ~$1.5\times10^8$ CFU/mL by 0.5 McFarland's barium sulphate standard solution. The dose of 1.0 ml containing $1.5\times10^8$ CFU of *E. coli* was given per bird in group-IV, V and VI orally by crop gavage on Day $14^{th}$. Livability was calculated based on recorded mortality that occurred during different experimental period.

### Enumeration of *E. coli* Counts

At day 21 and 28, three birds from each replicate as per treatment group were sacrificed. After evisceration of carcass, pre-caecal and caecal contents were taken aseptically and placed into separate marked sterile sample collection bags and stored at 4°C for further processing within 24 h. For bacteriological enumeration, pre-caecal and caecal contents were mixed properly, and homogenized mixture was prepared. To get decimal dilution, 0.1 gm (approximately 0.1 ml) sample was taken from homogenized mixture in sterile test tube and diluted with 900 µl phosphate buffer saline (PBS). From $10^{-4}$ to $10^{-7}$ dilution, 10 µl inoculum from each dilution were inoculated into separate marked sterile disposable petri-plates containing 10-15 ml sterile MacConkey agar. All the plates were incubated aerobically at 37°C for 24 h. Rapid lactose fermenting and dark pink colour colonies were considered as colonies of *E. coli*. The colonies were enumerated in a colony counter and the numbers were expressed as $Log_{10}$ CFU per gram of sample.

### Histomorphological Study of Small Intestine

After evisceration of carcass of birds sacrificed as above on day $21^{st}$ and $28^{th}$, 2 to 3 cm sections of duodenum, jejunum and ileum were removed, rinsed with PBS and cross-sectional lengths of 1 cm were fixed in 10% neutral buffered formalin solution. These were paraffin embedded, sectioned and stained with haematoxylin and eosin (H&E) stains (Luna, 1968) for histomorphological examination. The H&E-stained slides were observed under light microscope and all the measurements were made using an image analysis software (MagVision Imaging Software). The presence of intact lamina propria was used for villus selection to measure height and width of villi and length of crypt depth. For each sample, at least three regions with ten observations were examined, and the values were averaged to form a single observation. All samples from each bird were taken from the same area of each part of the small intestine.

### Gross and Histopathological Study

Post-mortem examination of each bird scarified from different treatment groups at different intervals was carried out and gross lesions if any were recorded. Tissues like thymus, bursa, spleen, liver, kidney and intestine were collected in tissue

collection bottles containing 10% neutral buffered formalin solution and processed by paraffin embedding technique for histopathological examination.

### Statistical Analysis

Completely randomized design and one-way-analysis of variance (ANOVA) were used to calculate and compare the means of different variables in control and treated broiler chicks by using SPSS statistics software (version 26.0). Significant differences ($p<0.05$) between different groups were analysed by Duncan's multiple range test.

## Results and Discussion

### Assessment of Antibacterial Effects

The significantly highest mean $Log_{10}$ CFU/g on 7$^{th}$ day post-infection of *E. coli* was observed in the group-IV followed by groups-VI, V, I, III and II, respectively. The significantly highest mean $Log_{10}$ CFU/g on 14$^{th}$ day post-infection of *E. coli* was observed in the group-IV followed by groups-I, VI, V, III and II, respectively (Table 1).

**Table 1:** Effect of dietary supplementation of cinnamon essential oil on *E. coli* counts ($Log_{10}$ CFU/g) in precaecal-caecal digesta of broiler chickens

| Group | $Log_{10}$ CFU/g | |
|---|---|---|
| | 7$^{th}$ day post-infection | 14$^{th}$ day post-infection |
| I | 7.36±0.009$^{c}$ | 7.96±0.003$^{e}$ |
| II | 7.04±0.019$^{a}$ | 7.20±0.013$^{a}$ |
| III | 7.23±0.012$^{b}$ | 7.71±0.007$^{b}$ |
| IV | 8.50±0.013$^{f}$ | 7.99±0.009$^{f}$ |
| V | 7.69±0.003$^{d}$ | 7.86±0.004$^{c}$ |
| VI | 7.81±0.005$^{e}$ | 7.91±0.003$^{d}$ |

Mean ± SEs (n=12) bearing different superscripts within a column differ significantly ($P<0.05$).

The results suggested that supplementation of cinnamon oil and chlortetracycline in broiler feed at sub-therapeutic level significantly reduced the bacterial count, but could not completely remove the pathogenic bacterial population at initial period of infection. However, on 14$^{th}$ day post-infection the caecal average bacterial counts of *E. coli* in birds of group-V and group-VI were significantly lower than group-I. Therefore, after certain period of time, cinnamon oil and chlortetracycline significantly reduced the caecal bacterial count and stabilised intestinal microbiota even after challenge of *E. coli* in birds. Similar significantly reduced the number of coliforms count from content of caecum in birds of *E. coli* + AGP group and birds of *E. coli* + NAT (Natural Feed Additives) treatment group as compared to birds of *E. coli* infected control group has been reported earlier (Manafi *et al.,* 2016). Several other researchers (Chowdhury *et al.,* 2018; Mehdipour and Afsharmanesh, 2018; Krauze *et al.,* 2020) also revealed significantly decreased counts of *E. coli* in small intestine of broilers supplemented with AGP and cinnamon oil/powder as compared to birds of control group.

### Histomorphological Study of Small Intestine

The villi hight and villi hight to crypt depth ratio of duodenum of birds on 21$^{st}$ day were significantly higher in group-III compared to all other groups (Fig. 1 to 3), whereas no significant difference was noticed between the villi width of group-III and VI. Further, the villi hight, villi width and villi hight to crypt depth ratio of duodenum on 21$^{st}$ day were found non-significant between the group-II and VI. The villi hight, villi width and villi hight to crypt depth ratio of jejunum of birds on 21$^{st}$ day were significantly higher in group-III compared to group-I, IV and V (Fig. 4, 5, 6), whereas no significant difference was noticed between the group-II and III. Also, the villi hight and villi hight to crypt depth ratio of jejunum on 21$^{st}$ day were found non-significant between group-I, V and VI. In ileum, the means of villi height, villi width and villi hight to crypt depth ratio on 21$^{st}$ day were significantly decreased in infected control group-IV as compared to all other groups (Fig. 7, 8, 9). The mean villi height of group-III was significantly increased as compared to groups-I, IV and V. The mean villi width of group-III was significantly increased as compared to all other groups. There was no significant difference noticed between the mean villi width of group-I and II. The mean villi height to crypt depth ratio of group-II and III were significantly increased as compared to groups-I, II, IV, V and VI (Table 2).

On 28$^{th}$ day, the mean villi height of duodenum in group-III was significantly increased as compared to groups-I, II, IV and V, whereas no significant difference was noticed between the mean villi height of group-II, V and VI. The mean villi width of duodenum was significantly decreased in infected control group-IV as compared to groups-II, III, V and VI, whereas no significant difference was noticed between group-I and IV. The mean villi height to crypt depth ratio in duodenum of group-III was significantly higher as compared to all other groups. The villi height and villi height to crypt depth ratio in jejunum were found higher in birds of group-VI as compared to birds of group-I, which was non-significant at 21$^{st}$ day of sacrifice (Fig. 10, 11). The mean villi height to crypt depth ratio in jejunum of group-III and VI was significantly increased as compared to groups-I, II, IV and V. In ileum, the mean villi height was significantly lower in infected control group-IV on 28$^{th}$ day as compared to groups-I, II, III, V and VI. However, there was no significant difference noticed between the mean villi height in rest of other groups. The significantly lower mean villi width was observed in the group-IV followed by groups-V, I, II and III. The mean villi height to crypt depth ratio of group-II and III was significantly increased as compared to groups-I, IV, V and VI (Table 3).

The findings of the present study agreed with earlier reports of various scientists, (Ahsan *et al.,* 2018; Chowdhury *et al.,* 2018; Krauze *et al.,* 2020) in broilers supplemented with phytogenic feed additive or AGP or cinnamon bark oil

**Table 2:** Effect of dietary supplementation of cinnamon oil on villi height (VH), villi width (VW) and villi height to crypt depth (CD) ratio of duodenum, jejunum and ileum in different experimental groups on 21st day (µm)

| Small intestine | | Group | | | | | |
|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI |
| **Duodenum** | Villi height | 2003.33±43.08$^{b}$ | 2224.67±43.77$^{c}$ | 2378.33±48.27$^{d}$ | 1852.24±29.28$^{a}$ | 2064.42±27.76$^{b}$ | 2211.50±36.56$^{c}$ |
| | Villi width | 236.83±4.28$^{b}$ | 242.00±4.40$^{bc}$ | 263.50±5.51$^{d}$ | 220.75±4.98$^{a}$ | 243.50±5.87$^{bc}$ | 255.58±4.35$^{cd}$ |
| | VH/CD ratio | 6.91±0.16$^{b}$ | 7.86±0.29$^{c}$ | 8.52±0.27$^{d}$ | 6.20±0.21$^{a}$ | 7.40±0.13$^{bc}$ | 7.88±0.22$^{c}$ |
| **Jejunum** | Villi height | 1611.42±40.57$^{b}$ | 1801.83±36.33$^{c}$ | 1825.25±40.89$^{c}$ | 1501.76±23.74$^{a}$ | 1668.17±31.61$^{b}$ | 1661.08±33.69$^{b}$ |
| | Villi width | 287.75±6.41$^{b}$ | 309.75±4.16$^{cd}$ | 324.75±6.84$^{d}$ | 260.33±5.39$^{a}$ | 297.17±5.37$^{bc}$ | 315.00±5.15$^{d}$ |
| | VH/CD ratio | 5.85±0.17$^{b}$ | 6.98±0.28$^{c}$ | 7.49±0.45$^{c}$ | 4.73±0.11$^{a}$ | 5.59±0.18$^{b}$ | 6.01±0.15$^{b}$ |
| **Ileum** | Villi height | 907.50±16.11$^{b}$ | 1055.17±19.31$^{cd}$ | 1098.00±18.50$^{d}$ | 805.47±14.49$^{a}$ | 1038.92±17.23$^{c}$ | 1061.67±22.13$^{cd}$ |
| | Villi width | 203.09±2.97$^{cd}$ | 212.05±5.11$^{d}$ | 241.03±5.92$^{e}$ | 167.42±3.00$^{a}$ | 179.02±2.17$^{b}$ | 195.75±3.52$^{c}$ |
| | VH/CD ratio | 4.28±0.13$^{b}$ | 5.16±0.14$^{c}$ | 5.56±0.15$^{d}$ | 3.36±0.10$^{a}$ | 3.98±0.10$^{b}$ | 4.02±0.14$^{b}$ |

Mean ± SEs (n=12) bearing different superscripts within the row differ significantly from each other (P<0.05).

**Table 3:** Effect of dietary supplementation of cinnamon oil on villi height (VH), villi width (VW) and villi height to crypt depth (CD) ratio of duodenum, jejunum and ileum in different experimental groups on 28th day (µm)

| Small intestine | | Group | | | | | |
|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI |
| **Duodenum** | Villi height | 2316.35±38.71$^{b}$ | 2463.50±35.36$^{c}$ | 2601.67±44.19$^{d}$ | 2157.13±41.24$^{a}$ | 2392.92±50.68$^{bc}$ | 2484.17±46.53$^{cd}$ |
| | Villi width | 261.67±6.65$^{a}$ | 297.38±6.42$^{cd}$ | 311.15±4.59$^{d}$ | 252.13±4.71$^{a}$ | 280.62±4.55$^{b}$ | 292.20±5.20$^{bc}$ |
| | VH/CD ratio | 9.20±0.16$^{b}$ | 11.90±0.32$^{d}$ | 12.99±0.31$^{e}$ | 8.10±0.23$^{a}$ | 10.33±0.33$^{c}$ | 11.53±0.27$^{d}$ |
| **Jejunum** | Villi height | 1890.92±42.86$^{a}$ | 2140.67±44.46$^{b}$ | 2200.92±48.49$^{b}$ | 1771.43±33.59$^{a}$ | 2080.00±49.71$^{b}$ | 2110.33±39.40$^{b}$ |
| | Villi width | 229.67±7.06$^{ab}$ | 238.33±4.00$^{ab}$ | 264.25±6.47$^{c}$ | 223.42±2.85$^{a}$ | 235.75±5.26$^{ab}$ | 245.98±6.38$^{b}$ |
| | VH/CD ratio | 6.38±0.25$^{ab}$ | 7.37±0.27$^{c}$ | 9.13±0.25$^{d}$ | 6.00±0.16$^{a}$ | 7.00±0.20$^{bc}$ | 8.51±0.21$^{d}$ |
| **Ileum** | Villi height | 1059.08±24.40$^{b}$ | 1239.50±23.51$^{c}$ | 1244.17±15.86$^{c}$ | 976.37±22.24$^{a}$ | 1181.89±22.48$^{c}$ | 1197.78±23.44$^{c}$ |
| | Villi width | 177.06±4.02$^{c}$ | 189.92±3.26$^{d}$ | 211.59±3.23$^{e}$ | 146.00±2.80$^{a}$ | 164.58±3.39$^{b}$ | 174.37±3.75$^{c}$ |
| | VH/CD ratio | 6.80±0.18$^{b}$ | 7.66±0.25$^{c}$ | 8.24±0.24$^{d}$ | 5.20±0.13$^{a}$ | 6.23±0.19$^{b}$ | 6.35±0.14$^{b}$ |

Mean ± SEs (n=12) bearing different superscripts within the row differ significantly from each other (P<0.05).

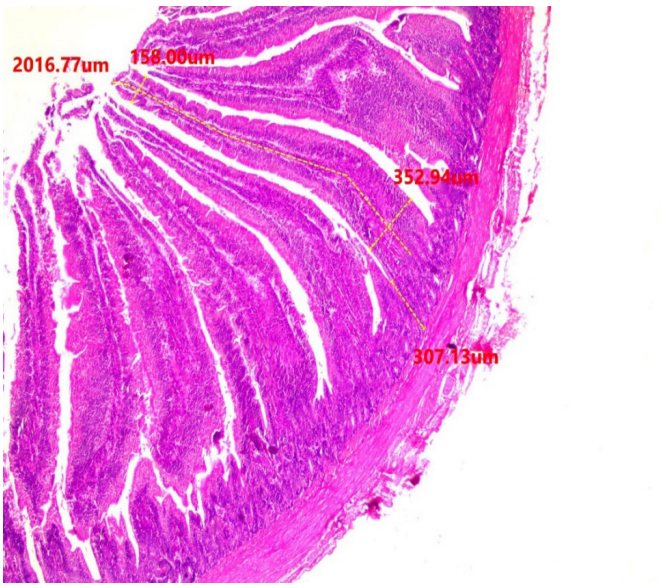


Fig.1 Section of duodenum from control group I showing normal height and width of villi and crypt depth on 21st day of experiment. (H&E x 48)

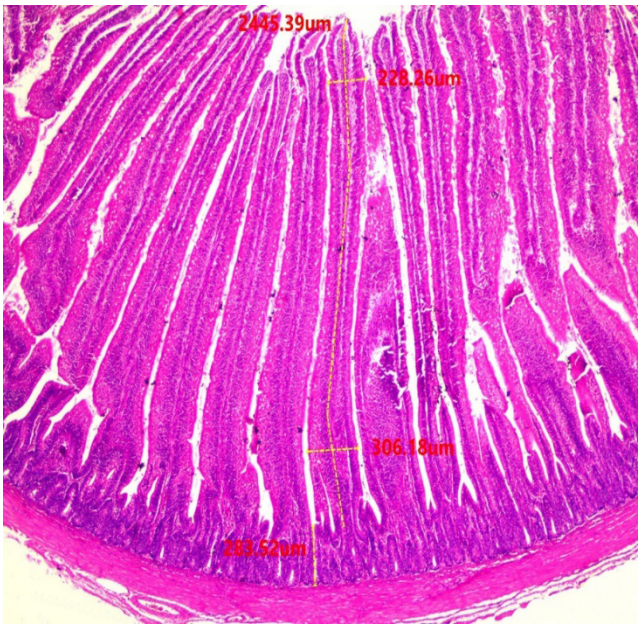


Fig. 2 Section of duodenum from group III showing increased height and width of villi and decreased crypt depth on 21st day of experiment. (H&E x 48)

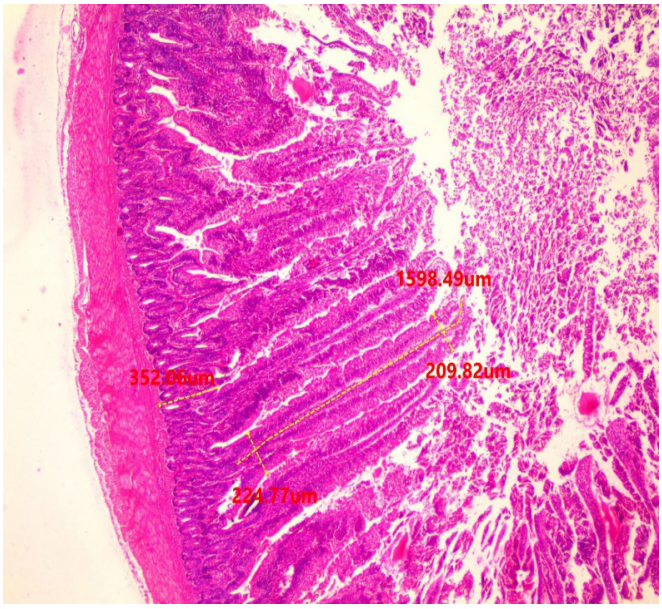


Fig.3 Section of duodenum from group IV showing decreased height and width of villi and increased crypt depth on 21st day of experiment. (H&E x 48)

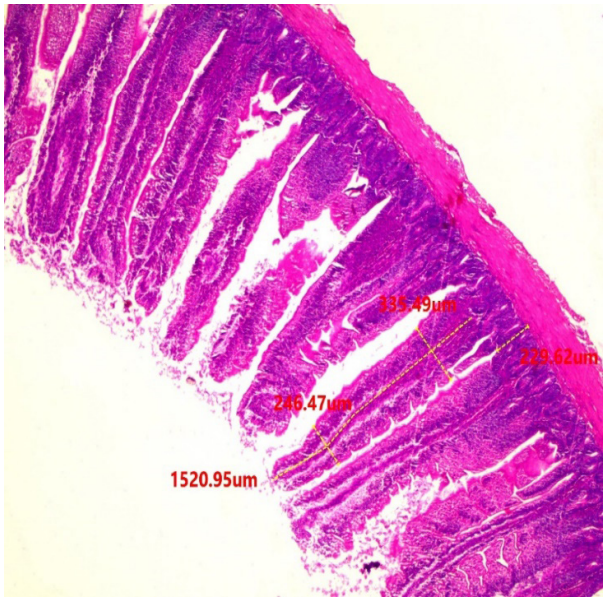


Fig.4 Section of jejunum from control group I showing normal height and width of villi and crypt depth on 21st day of experiment. (H&E x 48)

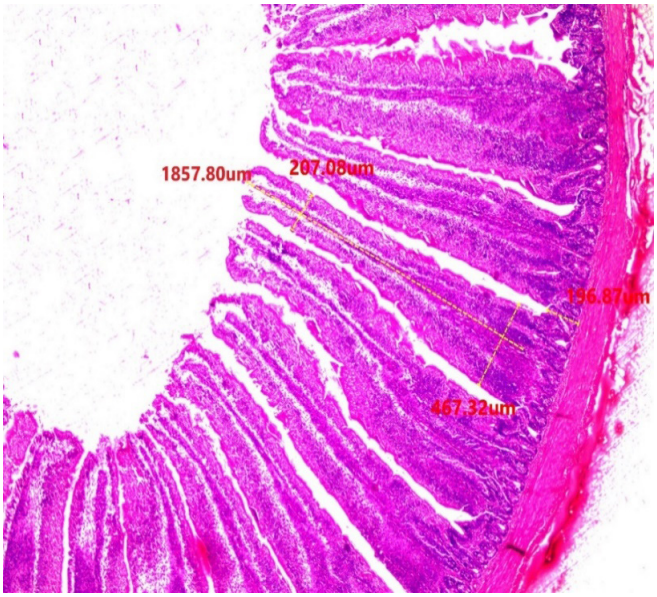


Fig.5 Section of jejunum from group IV showing increased height and width of villi and decreased crypt depth on 21st day of experiment. (H&E x 48)

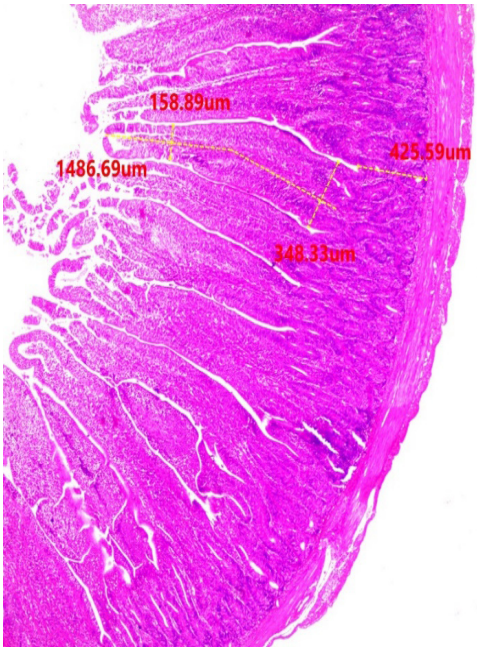


Fig.6 Section of jejunum from group IV showing decreased height and width of villi and increased crypt depth on 21st day of experiment. (H&E x 48)

or *Enterococcus faecium*, *Bacillus subtilis* and cinnamon oil as compared to birds of control group. However, in an earlier study, no significant difference on ileal villi height in birds of *E. coli* + AGP group and *E. coli* + NAT group was recorded as compared to birds of *E. coli* infected control group (Manafi *et al.,* 2016).

In the present study, the observations indicated that the supplementation of cinnamon oil in broiler diets increased the villi height (VH), villi width (VW) and villi height to crypt depth ratio in the duodenum, jejunum and ileum of broilers even in the presence of *E. coli* infection as compared to birds of control and chlortetracycline group. These effects of essential oils seen during the period of 21st and 28th day of sacrifice without any variation can be attributed to their antioxidant and antibacterial properties. Cinnamaldehyde has beneficial effects through intracellular antioxidant action for the protection of intestinal villi (Jamroz *et al.*, 2005). The EO phenolic group may act as a donor of hydrogen ions to exhibit antioxidant activity (Windisch *et al.*, 2008).

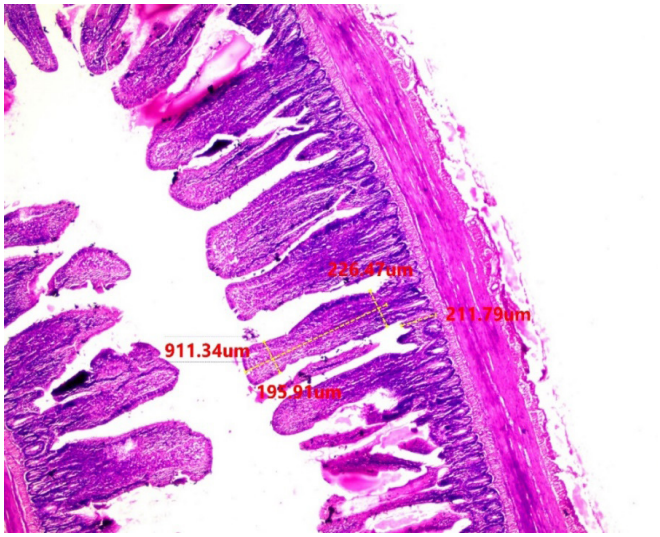


Fig.7 Section of ileum from control group I showing normal height and width of villi and crypt depth on 21st day of experiment. (H&E x 48)

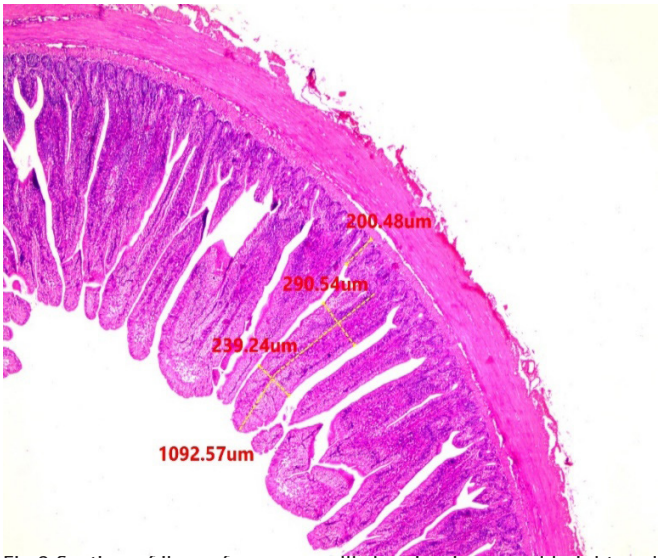


Fig.8 Section of ileum from group III showing increased height and width of villi and decreased crypt depth on 21st day of experiment. (H&E x 48)

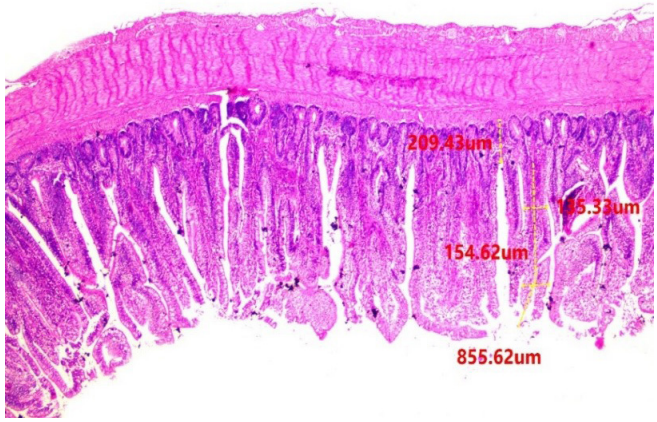


Fig.9 Section of ileum from control group IV showing decreased height and width of villi and increased crypt depth on 21st day of experiment. (H&E x 48)

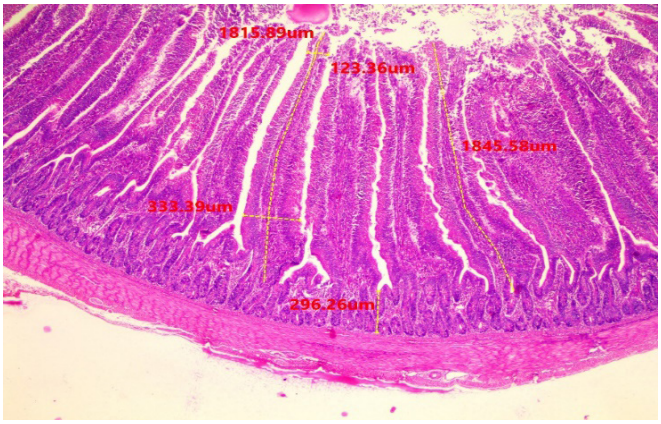


Fig.10 Section of jejunum from control group I showing normal height and width of villi and crypt depth on 28th day of experiment. (H&E x 48)

Furthermore, essential oils have antibacterial properties that help in the homeostasis of the gut microbiome and the prevention of pathogens associated damage to the intestinal villi (Jamroz *et al.*, 2006). Increased height and width of intestinal villi in birds supplemented with cinnamon oil ultimately enlarges the absorptive surface of the gut and enhances nutrient absorption. Moreover, higher villus height to crypt depth ratio results in a decreased turnover rate of the intestinal epithelium which leads to a lower maintenance requirement and finally can result in a higher growth efficiency of the birds.

***Livability:*** There was no mortality recorded in any of the experimental groups during the study period indicating that per oral *E. coli* infection was not fatal to birds, as well as the fact that all normal management methods for bird raising were followed throughout the experiment. Furthermore, it's possible that cinnamon essential oil supplementation has no negative effects in birds at the doses used in their diet.

## Gross and Histopathological Study

The small intestine of a few birds from the infected control group-IV showed congested blood vessels and a presence of catarrhal exudates in lumen on 21st day of sacrifice. However, there were no appreciable gross lesions noticed in birds of group-IV on 28th day of sacrifice. Also, there were no appreciable gross pathological lesions noticed in the small intestine of birds of treatment groups-I, II, III, V and VI at any interval of sacrifice. Moreover, there were no appreciable gross lesions noticed in the other organs, *i.e.,* liver, kidney, heart, bursa of fabricius, thymus and spleen in any of treatment groups at any interval of sacrifice. Similar observations were reported by earlier workers (Tonu *et al.,* 2011; Yun *et al.,* 2018) in the small intestine of naturally affected chickens with *E. coli* and in liver and kidney of rats. However, Pourbakhsh *et al.* (1997) and Elmenawey *et al.* (2019) reported moderate to severe lesions of air sacculitis,

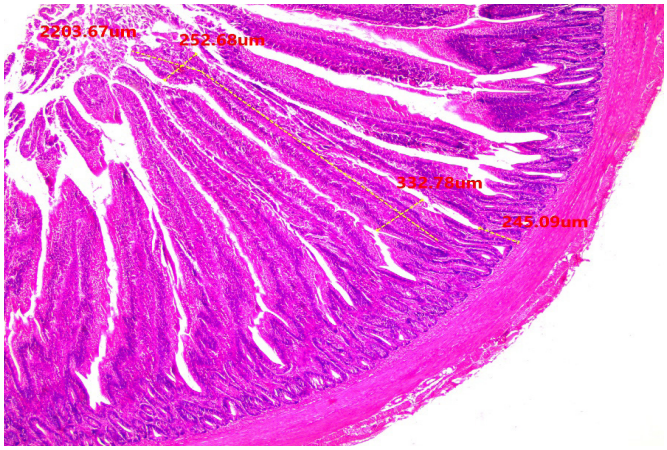


Fig.11 Section of jejunum from group VI showing increased height and width of villi and decreased crypt depth on 28th day of experiment compared to group I. (H&E x 48)

perihepatitis, pericarditis and splenic hypertrophy in experimentally affected chickens with *E. coli*.

On histopathological examinations of small intestine, a few birds of group-IV revealed mild degenerations and necrosis in the epithelial lining of intestinal villi along with congestion and infiltration of inflammatory cells in the mucosa on 7th day post-infection. Furthermore, the lesions in group-IV reduced 14 days after infection and were comparable to the birds of other groups. There were no remarkable histopathological lesions noted in small intestine in any of the other experimental groups at any interval of sacrifice. Sections of liver, heart, spleen, kidney, thymus and bursa of fabricius did not show histopathological changes in any of the groups at any interval of sacrifice including the control.

The overall gross and histopathological lesions showed mild degenerative and inflammatory changes in small intestine. Earlier scientists have also reported necrosis and desquamation of mucosal epithelium with severe infiltration of leukocytes mainly heterophils, lymphocytes and macrophages in the submucosa of the duodenal wall in naturally affected chickens with *E. coli* (Tonu *et al.*, 2011). Cinnamon extract did not produce any altered histopathological lesions in liver and kidney of rats (Yun *et al.,* 2018). However, inflammatory cell infiltration, serous to fibrinous exudate and cellular debris on serosal surfaces were reported in spleen, liver and airsacs in experimentally affected chickens with *E. coli* (Pourbakhsh *et al.,* 1997), while lymphoid cell proliferation in bursa of Fabricius and thymus in the groups treated with essential oil as compared to control group was observed by others (Awaad *et al.,* 2010; Parmar, 2020; Goswami, 2020).

## Conclusions

The findings of the study indicated that dietary supplementation of cinnamon oil in broiler chicks significantly decreased counts of *Escherichia coli* ($Log_{10}$ CFU/g) in precaecal-caecal digesta at 7th and 14th day post-infection showing its potential to stabilise intestinal microbiota even after challenge of *E. coli* in birds. However, supplementation of chlortetracycline in broiler feed was more effective than cinnamon oil in the term of *in-vivo* antibacterial activity against *E. coli*. Cinnamon oil supplementation comparatively increased the villi height, villi width and villi height to crypt depth ratio in the small intestine of birds and was more effective than chlortetracycline. This change enlarges the absorptive surface of the gut and enhances nutrient absorption resulting in a lower maintenance requirement and a higher growth efficiency of the birds. Supplementation of cinnamon oil at 400 mg/kg feed had no adverse effects on broiler birds. Thus, cinnamon oil can be used as a natural and safe growth promoter in broiler diet without any ill-effects on the health of broiler birds.

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